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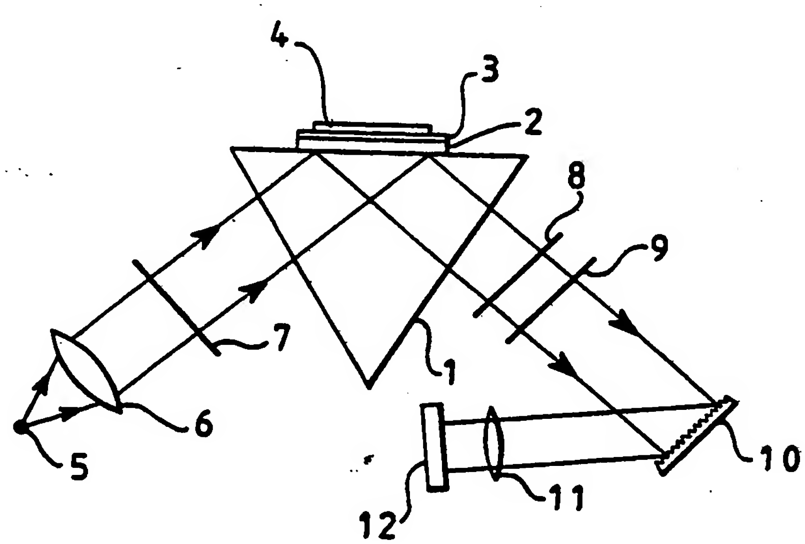
(54) Title: ANALYTICAL DEVICE WITH POLYCHROMATIC LIGHT SOURCE



(57) Abstract

Apparatus for the determination of a chemical or biochemical species comprises a resonant optical biosensor (1-4) disposed in a light path between a source (5) of polychromatic collimated light and a detector (10, 12) adapted to monitor some characteristic of the light. The detector (10, 12) includes a wavelength-dispersive element (10). The source (5) of polychromatic light is preferably a point light source, e.g. a semi-conductor light source such as a light-emitting diode, and the wavelength-dispersive element (10) may be a grating or a holographic lens.

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Title : Analytical Device with Polychromatic Light Source

This invention relates to sensors, especially those termed biosensors, ie devices for the analysis of biologically active species such as antigens and antibodies in samples of biological origin. In particular, the invention relates to biosensors based on resonant optical phenomena, eg surface plasmon resonance or resonant attenuated or frustrated total internal reflection.

Many devices for the automatic determination of biochemical analytes in solution have been proposed in recent years. Typically, such devices (biosensors) include a sensitised coating layer which is located in the evanescent region of a resonant field. Detection of the analyte typically utilizes optical techniques such as, for example, surface plasmon resonance (SPR), and is based on changes in the thickness and/or refractive index of the coating layer resulting from interaction of that layer with the analyte. This causes a change, eg in the angular position of the resonance.

Other optical biosensors include a waveguide in which a beam of light is propagated. The optical characteristics of the device are influenced by changes occurring at the surface of the waveguide. One form of optical biosensor is based on frustrated total reflection. The principles of frustrated total reflection (FTR) are well-known; the technique is described, for example, by Bosacchi and Oehrle [Applied Optics (1982), 21, 2167-2173]. An FTR device for use in immunoassay is disclosed in European Patent Application No 2205236A and comprises a cavity layer bounded on one side by the sample under investigation and on the other side by a spacer layer which in turn is mounted on a substrate. The substrate-spacer layer interface is irradiated with monochromatic radiation such that total reflection occurs, the associated evanescent field penetrating through the spacer layer. If the thickness of the spacer layer is correct and the incident parallel wave

vector matches one of the resonant mode propagation constants, the total reflection is frustrated and radiation is coupled into the cavity layer. The cavity layer must be composed of material which has a higher refractive index than the spacer layer and which is transparent at the wavelength of the incident radiation.

In devices of this kind, the position of resonance may be monitored by scanning the angle at which monochromatic light is incident on the sensor, or by scanning the wavelength of light incident on the sensor at a constant angle. In the former case, the scanning of angle may be performed either sequentially or simultaneously ie by varying the angle of incidence of a parallel beam of light or by simultaneously irradiating over a range of angles using a fan-shaped beam of light as described (in connection with SPR) in European Patent Application No 0305109A. In the first configuration, prior proposals have involved a single-channel detector which is mechanically scanned over a range of angles; this necessitates synchronisation of the movement of the light source and the detector. In the second configuration, in which a range of angles is irradiated simultaneously, it is generally necessary to use a multi-channel detector having angular resolution. This leads to relatively high manufacturing costs.

Where the wavelength is scanned at constant angle of incidence, similar disadvantages occur and, in addition, the necessary polychromatic light source may be expensive.

There has now been devised an apparatus involving the use of a resonant optical sensor for the determination of a chemical or biochemical species, which overcomes or substantially mitigates some or all of the disadvantages of the prior art arrangements described above.

According to the invention, there is provided apparatus for the determination of a chemical or biochemical species,

comprising a resonant optical biosensor disposed in a light path between a source of polychromatic collimated light and a detector adapted to monitor some characteristic of the light, the detector including a wavelength-dispersive element.

The apparatus according to the invention is advantageous primarily in that it is of relatively simple and inexpensive construction, eg due to the absence of moving parts.

The source of polychromatic light is preferably a point light source, ie a source of sufficiently small physical size to provide, with simple collimating optics, good collimation of the incident beam.

The light source may be a filament light source with a small aperture. Alternatively, and preferably, the light source is a semiconductor light source, eg a light emitting diode.

By `polychromatic light' is meant light which has a bandwidth sufficiently broad to encompass the wavelength at which resonance occurs. Where an LED is used, for example, the bandwidth is typically a few tens of nanometres.

In this context, 'light' may include not only visible light but also wavelengths above and below this range, eg in the ultra-violet and infra-red.

The wavelength-dispersive element may be a grating. In this case, the pitch of the grating is preferably close to the wavelength of light at the high end of the polychromatic bandwidth. This maximises the separation of wavelengths across the bandwidth. Alternatively, the wavelength-dispersive element may be a holographic lens. This simplifies the optics since no additional focussing lens is required.

The characteristic of the light which is monitored may be any characteristic which changes at resonance, eg the phase of

reflected radiation or, in some cases, the intensity.

The sensor is preferably an FTR sensor. Such a sensor will generally include an optical structure comprising

a) a cavity layer of transparent dielectric material of refractive index n_3,

b) a dielectric substrate of refractive index n_1, and

c) interposed between the cavity layer and the substrate, a dielectric spacer layer of refractive index n_2.

In use, the interface between the substrate and the spacer layer is irradiated with light such that internal reflection occurs. Resonant propagation of a guided mode in the cavity layer will occur, for a given angle of incidence, at a particular wavelength of the incident radiation.

The wavelength at which the resonant effect occurs depends on various parameters of the sensor device, such as the refractive indices and thicknesses of the various layers. In general, it is a pre-requisite that the refractive index n_3 of the cavity layer and the refractive index n_1 of the substrate should both exceed the refractive index n_2 of the spacer layer. Also, since at least one mode must exist in the cavity to achieve resonance, the cavity layer must exceed a certain minimum thickness.

The cavity layer is preferably a thin-film of dielectric material. Suitable materials for the cavity layer include zirconium dioxide, titanium dioxide, aluminium oxide and tantalum oxide.

The cavity layer may be prepared by known techniques, eg vacuum evaporation, sputtering, chemical vapour deposition or in-diffusion.

The dielectric spacer layer must have a lower refractive index than both the cavity layer and the substrate. The layer may,

for example, comprise an evaporated or sputtered layer of magnesium fluoride. Other suitable materials include lithium fluoride and silicon dioxide. Apart from the evaporation and sputtering techniques mentioned above, the spacer layer may be deposited on the substrate by a sol-gel process, or be formed by chemical reaction with the substrate.

The sol-gel process is particularly preferred where the spacer layer is of silicon dioxide.

The refractive index of the substrate (n_1) must be greater than that (n_2) of the spacer layer but the thickness of the substrate is generally not critical.

By contrast, the thickness of the cavity layer must be so chosen that resonance occurs within an appropriate range of coupling angles. The spacer layer will typically have a thickness of the order of several hundred nanometres, say from about 200nm to 2000nm, more preferably 500 to 1500nm, eg 1000nm. The cavity layer typically has a thickness of a few tens of nanometres, say 10 to 200nm, more preferably 30 to 150nm, eg 100nm.

It is particularly preferred that the cavity layer has a thickness of 30 to 150nm and comprises a material selected from zirconium dioxide, titanium dioxide, tantalum oxide and aluminium oxide, and the spacer layer has a thickness of 500 to 1500nm and comprises a material selected from magnesium fluoride, lithium fluoride and silicon dioxide, the choice of materials being such that the refractive index of the spacer layer is less than that of the cavity layer.

Preferred materials for the cavity layer and the spacer layer are tantalum oxide and silicon dioxide respectively.

At resonance, the incident light is coupled into the cavity layer by FTR, propagates a certain distance along the cavity

layer, and couples back out (also by FTR). The propagation distance depends on the various device parameters but is typically of the order of 1 or 2mm.

At resonance the reflected light will undergo a phase change, and it is this which may be detected. Alternatively, as described in our co-pending International Patent Application No PCT/GB91/01161 the cavity layer and/or spacer layer may absorb at resonance, resulting in a reduction in the intensity of the reflected light.

For use in the determination of biochemical species, the surface of the sensor, ie the surface of the cavity layer in the case of an FTR sensor, will generally be sensitised by having biomolecules, eg specific binding partners for the analyte(s) under test, immobilised upon it. The immobilised biochemicals may be covalently bound to the sensor surface by methods which are well known to those skilled in the art.

The invention will now be described in more detail, by way of illustration only, with reference to the accompanying drawings in which

Figure 1 is a schematic view (not to scale) of an apparatus according to the invention,

Figure 2 depicts the dependence of the intensity of the detected light on the wavelength, and

Figure 3 is a schematic view of part of a second embodiment of an apparatus according to the invention.

Referring first to Figure 1, a biosensor comprises a glass prism 1 coated over an area of its base with a first coating 2 of magnesium fluoride and a second coating 3 of titanium dioxide. The prism 1 and first and second coatings 2,3 together constitute a resonant optical structure, the first

coating 2 acting as a spacer layer and the second coating 3 as a cavity layer. The first coating 2 has a thickness of approximately 1000nm and the second coating 3 a thickness of approximately 100nm.

Immobilised on the surface of the second coating 3 is a layer 4 of immobilised biochemicals, which act as specific binding partner for the analyte under test.

The interface between the base of the prism 1 and the first coating 2 is irradiated by a beam of polychromatic light from a light emitting diode (LED) 5. Light from the LED 5 has a bandwidth of about 50nm, centred at about 640nm. Light from the LED 5 is collimated by a lens 6 and passes through a polariser 7.

The polariser 7 is arranged to produce linearly polarised light with two components : transverse electric (TE) and transverse magnetic (TM). The polariser is set at 45° to the TE and TM transmission axes and thus provides equal components of TE and TM light.

All the light incident on the interface between the base of the prism 1 and the first coating 2 is reflected, resonance being detected as a change of phase of the reflected light.

The reflected light is passed through a compensator 8 to a polarisation analyser 9. The compensator 8 is manually adjusted to remove any phase difference which is introduced into the TE and TM components on reflection and by birefringence in the optical path.

The analyser 9 is arranged at 90° to the polariser 7. The TE and TM components are interfered at the analyser to allow the phase change to be detected. Off resonance both components undergo a similar phase shift on reflection and the relative phase between the components is adjusted by the compensator 8

to give zero transmission through the analyser 9. This will apply for all wavelengths except near resonance. Near resonance of either component, the phase shift between the TE and TM components will vary rapidly with wavelength, resulting in maximum throughput of light through the analyser 9 at resonance.

Light passing through the analyser 9 is diffracted by a grating 10 and focussed by a lens 11 onto a detector 12.

In use, polychromatic light is incident on the interface between the base of the prism 1 and the first coating layer 2 at a fixed angle of incidence. At that angle, resonance occurs for one particular wavelength. At off-resonance wavelengths, no light intensity is detected at the detector 12; progressively closer to resonance, the detected light intensity increases and then falls.

When the layer of immobilised biochemicals 4 is contacted with a sample containing the analyte under test, specific binding occurs between the biochemicals and the analyte molecules, resulting in a change in the refractive index in the vicinity of the surface of the device. This in turn results in a shift in the position of the resonance. Figure 2 shows a plot of the measured signal intensity against wavelength before and (dotted line) after complexation of the immobilised biochemicals with the analyte.

In the embodiment shown in Figure 3, the grating 10 and lens 11 are replaced by a holographic lens 13 which simultaneously disperses and focusses the reflected light onto the detector 12.

Claims

1. Apparatus for the determination of a chemical or biochemical species, comprising a resonant optical biosensor (1-4) disposed in a light path between a source (5) of polychromatic collimated light and a detector (10,12) adapted to monitor some characteristic of the light, the detector (10,12) including a wavelength-dispersive element (10).

2. Apparatus as claimed in Claim 1, wherein the source (5) of polychromatic light is a point light source.

3. Apparatus as claimed in Claim 1 or Claim 2, wherein the light source is a filament light source with a small aperture.

4. Apparatus as claimed in Claim 1 or Claim 2, wherein the light source (5) is a semiconductor light source, eg a light emitting diode.

5. Apparatus as claimed in any preceding claim, wherein the wavelength-dispersive element (10) is a grating.

6. Apparatus as claimed in Claim 5, wherein the pitch of the grating (10) is close to the wavelength of light at the high end of the polychromatic bandwidth.

7. Apparatus as claimed in any one of Claims 1 to 5, wherein the wavelength-dispersive element is a holographic lens.

8. Apparatus as claimed in any preceding claim, wherein the biosensor is an FTR sensor comprising
a) a cavity layer (3) of transparent dielectric material of refractive index n_3,
b) a dielectric substrate (1) of refractive index n_1, and
c) interposed between the cavity layer (3) and the substrate (1), a dielectric spacer layer (2) of refractive index n_2.

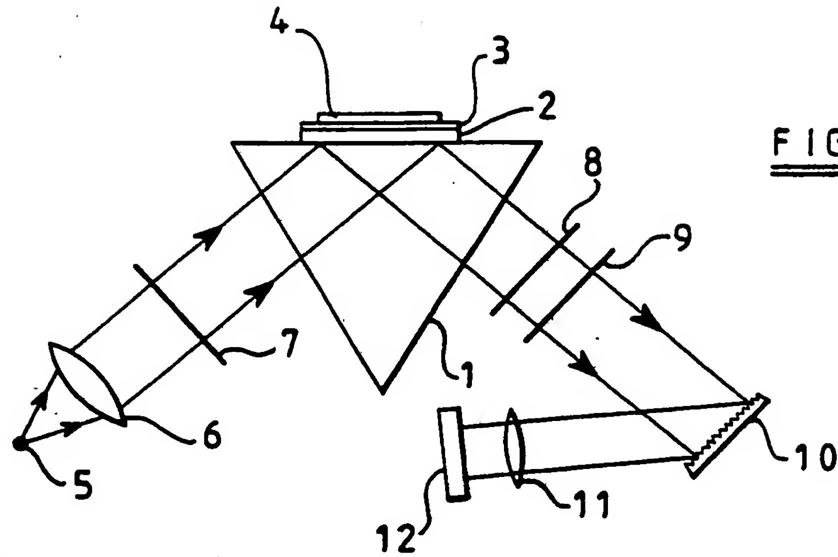

FIG 1

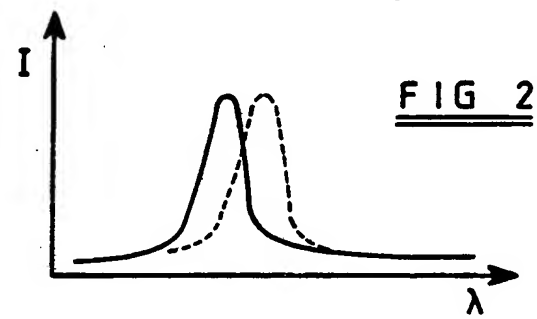

FIG 2

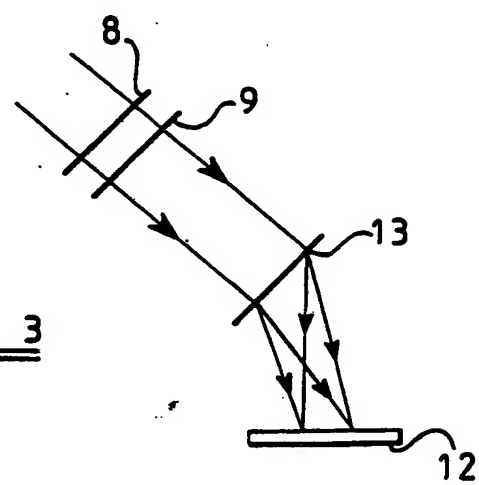

FIG 3

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 93/00026

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 G01N21/55

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	G01N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	EP,A,0 257 955 (THORN EMI) 2 March 1988 see column 1, line 3 - line 5 see column 3, line 40 - line 64; claim 1; figure 4	1,2,5
Y	---	4,7,8
Y	GB,A,2 197 068 (STC) 11 May 1988 see page 1, line 51 - line 65; figure 1 ---	4
Y	EP,A,0 452 095 (HUGHES AIRCRAFT) 16 October 1991 see claim 1 ---	7
P,Y	WO,A,9 203 720 (FISON HOUSE) 5 March 1992 cited in the application see claim 1; figure --- -/--	8

° Special categories of cited documents : [10]

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IV. CERTIFICATION

1

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
07 APRIL 1993	22.04.93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	KRAMETZ E.M.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	WO,A,9 110 122 (BATTELLE) 11 July 1991 see page 11, line 26 - line 10; figure 1	1,5
A	EP,A,0 305 109 (AMERSHAM) 1 March 1989 cited in the application see column 5, line 28 - column 6, line 46; figure 3	1

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9300026
SA 68555

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 07/04/93
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		US-A- 4844613	04-07-89
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		JP-A- 4225124	14-08-92
WO-A-9203720	05-03-92	None	
WO-A-9110122	11-07-91	US-A- 5082629	21-01-92
		EP-A- 0507883	14-10-92
EP-A-0305109	01-03-89	AU-B- 602423	11-10-90
		AU-A- 2110988	23-02-89
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		US-A- 4997278	05-03-91

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